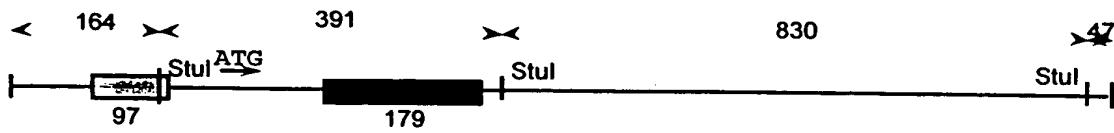
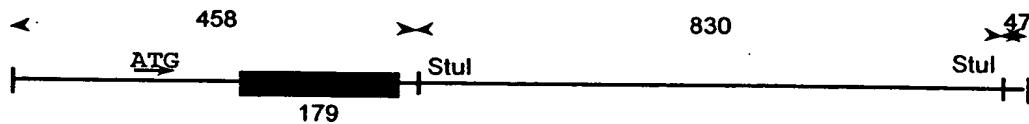


Figure 1

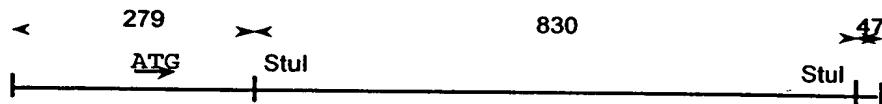
Full Length:



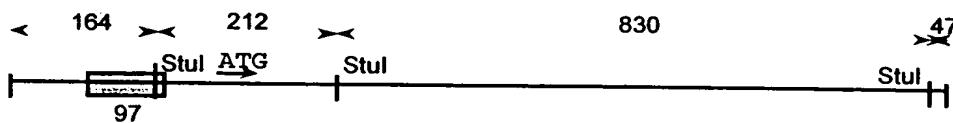
Properly Spliced:



Downstream Missplice:



Unsplice Intron and Downstream Missplice:



■ Upstream Splice Site (part of the expression vector)

■ 'Weird' Splice Site (found in the Gcc cDNA sequence)

Figure 2

r_rAG/ qua aqu Consensus Donor

5' - AAG CCG TTG AGT AGG/ GTC AGC ATC ATG GCT GGC AGC CTC AC 160-

a) unmodified Gcc

Lys Pro Leu Ser Arg Val Ser Ile Met Ala Gly Ser Leu Thr 26

5' - AAG CCG TTG AGT AGA GTC TCC ATC ATG GCT GGC AGC CTC AC 160-

* * *

b) "modified Gcc"

yyy yyy yyy ync ag/G

Consensus Acceptor

5' - TTT CCT GCC CTT GGT ACC TTC AG/C CGC TAT GAG AGT ACA C 340-

a) unmodified Gcc

Phe Pro Ala Leu Gly Thr Phe Ser Arg Tyr Glu Ser Thr Arg

83

5' TTT CCT GCC CTG GGA ACA TTT TCC CGC TAT GAG AGT ACA C 340-

* * * * *

Figure 3 (continued on next page)

b) modified Gcc

Product of Alternatively Spliced mRNA transcript from the unmodified Gcc cDNA:

NOTE:

Nucleotides associated with RNA splice-sites are underlined. Those believed to be of most importance to splice site-recognition are double underlined.

" / " in Consensus Splice Sequence indicates splice site. " * " under a particular nucleotide indicates a base changed in the site-directed mutagenesis procedure.

r = C or A, y = c or u, and n = any.

Figure 3 (continued on next page)

Partial produce of the cryptic splicing of Gcc cDNA

5' - AAG CCG TTG AGT AGG CCG CTA TGA GAG TAC AC

Lys Pro Leu Ser Arg¹⁶ Pro Leu STOP²⁰ !!

Figure 3 (continued)

10 123456789012345	20 678901234567890	30 123456789012345	40 678901234567890	50 678901234567890	60
<hr/>					
NotI					
NGCGGCCGCTTAGCT	TGACTTAAGAAGGCC	GACGCCATGGAGTTT	TCAAGTCCTCCAGA		60
			MetGluPhe	SerSerProSerArg	
GAGGAATGTCCAAG	CCTTGAGTAGAGTC	TCCATCATGGCTGGC	AGCCTCACAGGTTTG		120
GluGluCysProLys	ProLeuSerArgVal	SerIleMetAlaGly	SerLeuThrGlyLeu		
CTTCTACTTCAGGCA	GTGTCGTGGCATCA	GGTGCCCGCCCCCTGC	ATCCCTAAAGCTTC		180
LeuLeuLeuGlnAla	ValSerTrpAlaSer	GlyAlaArgProCys	IleProLysSerPhe		
GGCTACAGCTCGGTG	GTGTGTCTGCAAT	GCCACATACTGTGAC	TCCTTGACCCCCCG		240
GlyTyrSerSerVal	ValCysValCysAsn	AlaThrTyrCysAsp	SerPheAspProPro		
ACCTTCCTGCCCTG	GGAACATTTCCCGC	TATGAGAGTACACGC	AGTGGGCGACGGATG		300
ThrPheProAlaLeu	GlyThrPheSerArg	TyrGluSerThrArg	SerGlyArgArgMet		
GAGCTGAGTATGGGG	CCCATCCAGGCTAAT	CACACGGGCACAGGC	CTGCTACTGACCCTG		360
GluLeuSerMetGly	ProIleGlnAlaAsn	HisThrGlyThrGly	LeuLeuLeuThrLeu		
CAGCCAGAACAGAAG	TTCCAGAAAGTGAAG	GGATTGGAGGGGCC	ATGACAGATGCTGCT		420
GlnProGluGlnLys	PheGlnLysValLys	GlyPheGlyGlyAla	MetThrAspAlaAla		
GCTCTAACATCCTT	GCCCTGTCACCCCT	GCCCAAATTTGCTA	CTTAAATCGTACTTC		480
AlaLeuAsnIleLeu	AlaLeuSerProPro	AlaGlnAsnLeuLeu	LeuLysSerTyrPhe		
TCTGAAGAAGGAATC	GGATATAACATCATC	CGGGTACCCATGGCC	AGCTGTGACTTCTCC		540
SerGluGluGlyIle	GlyTyrAsnIleIle	ArgValProMetAla	SerCysAspPheSer		
ATCCGCACCTACACC	TATGCAGACACCCCT	GATGATTCCAGTTG	CACAATTCAGCCTC		600
IleArgThrTyrThr	TyrAlaAspThrPro	AspAspPheGlnLeu	HisAsnPheSerLeu		
CCAGAGGAAGATAACC	AAGCTCAAGATAACC	CTGATTCAACCGAGCC	CTGCAGTTGGCCAG		660
ProGluGluAspThr	LysLeuLysIlePro	LeuIleHisArgAla	LeuGlnLeuAlaGln		
CGTCCCCTTCACTC	CTGCCAGCCCCCTGG	ACATCACCCACTTGG	CTCAAGACCAATGGA		720
ArgProValSerLeu	LeuAlaSerProTrp	ThrSerProThrTrp	LeuLysThrAsnGly		

Figure 4A (continued on next page)

10	20	30	40	50	60
<u>123456789012345</u>	<u>678901234567890</u>	<u>123456789012345</u>	<u>678901234567890</u>		

GCGGTGAATGGGAAG	GGGTCACTCAAGGGA	CAGCCCGAGACATC	TACCACCAGACCTGG		780
AlaValAsnGlyLys	GlySerLeuLysGly	GlnProGlyAspIle	TyrHisGlnThrTrp		
GCCAGATACTTGATGCC	TATGCTGAGCACAAAG	TTACAGTTCTGGGCA			840
AlaArgTyrPheVal	LysPheLeuAspAla	TyrAlaGluHisLys	LeuGlnPheTrpAla		
GTGACAGCTGAAAAT	GAGCCTCTGCTGGG	CTGTTGAGTGGATAC	CCCTTCCAGTGCCTG		900
ValThrAlaGluAsn	GluProSerAlaGly	LeuLeuSerGlyTyr	ProPheGlnCysLeu		
GGCTTCACCCCTGAA	CATCAGCGAGACTTA	ATTGCCCGTGACCTA	GGTCCTACCCCTCGCC		960
GlyPheThrProGlu	HisGlnArgAspLeu	IleAlaArgAspLeu	GlyProThrLeuAla		
AACAGTACTCACCAC	AATGTCCGCCTACTC	ATGCTGGATGACCAA	CGCTTGCTGCTGCC		1020
AsnSerThrHisHis	AsnValArgLeuLeu	MetLeuAspAspGln	ArgLeuLeuLeuPro		
CACTGGCAAAGGTG	GTACTGACAGACCCA	GAAGCAGCTAAATAT	GTTCATGGCATTGCT		1080
HisTrpAlaLysVal	ValLeuThrAspPro	GluAlaAlaLysTyr	ValHisGlyIleAla		
GTACATTGGTACCTG	GACTTTCTGGCTCCA	GCCAAAGCCACCTA	GGGGAGACACACCCGC		1140
ValHisTrpTyrLeu	AspPheLeuAlaPro	AlaLysAlaThrLeu	GlyGluThrHisArg		
CTGTTCCCCAACACC	ATGCTCTTGCCCTCA	GAGGCCTGTGTGGC	TCCAAGTTCTGGGAG		1200
LeuPheProAsnThr	MetLeuPheAlaSer	GluAlaCysValGly	SerLysPheTrpGlu		
CAGAGTGTGCGGCTA	GGCTCCTGGATCGA	GGGATGCAGTACAGC	CACAGCATCATCACG		1260
GlnSerValArgLeu	GlySerTrpAspArg	GlyMetGlnTyrSer	HisSerIleIleThr		
AACCTCCTGTACCAT	GTGGTCGGCTGGACC	GACTGGAACCTTGCC	CTGAACCCCGAAGGA		1320
AsnLeuLeuTyrHis	ValValGlyTrpThr	AspTrpAsnLeuAla	LeuAsnProGluGly		
GGACCCAATTGGGTG	CGTAACCTTGTGAC	AGTCCCATCATTGTA	GACATCACCAAGGAC		1380
GlyProAsnTrpVal	ArgAsnPheValAsp	SerProIleIleVal	AspIleThrLysAsp		
ACGTTTACAAACAG	CCCATGTTCTACCAAC	CTTGGCCATTTCAGC	AAGTTCATTCCTGAG		1440
ThrPheTyrLysGln	ProMetPheTyrHis	LeuGlyHisPheSer	LysPheIleProGlu		

Figure 4A (continued on next page)

	10	20	30	40	50	60	
	123456789012345	678901234567890	123456789012345	678901234567890			
GGCTCCCAGAGAGTG	GGGCTGGTGCAGT	CAGAAGAACGACCTG	GACGCAGTGGCATTG				1500
GlySerGlnArgVal	GlyLeuValAlaSer	GlnLysAsnAspLeu	AspAlaValAlaLeu				
ATGCATCCCGATGGC	TCTGCTGTTGTGGTC	GTGCTAAACCGCTCC	TCTAAGGATGTGCCT				1560
MetHisProAspGly	SerAlaValValVal	ValLeuAsnArgSer	SerLysAspValPro				
CTTACCACATCAAGGAT	CCTGCTGTGGGCTTC	CTGGAGACAATCTCA	CCTGGCTACTCCATT				1620
LeuThrIleLysAsp	ProAlaValGlyPhe	LeuGluThrIleSer	ProGlyTyrSerIle				
CACACCTACCTGTGG	CATCGCCAGTGTGG	AGCAGATACTCAAGG	AGGCACTGGGCTCAG				1680
HisThrTyrLeuTrp	HisArgGln						
CCTGGGCATTAAAGG	GACAGAGTCAGCGAA	TTCTGCAGATATCCA	TCACACTGGCGGCCG	NotI			1740

Figure 4A(continued)

10	20	30	40	50	60
123456789012345	678901234567890	123456789012345	678901234567890		

Not I

<u>NGCGGCCGCTTAGCT</u> TGACTTAAGAAGGCC GACGCCATGGAGTT TCAAGTCCTCCAGA <u>MetGluPhe</u> <u>SerSerProSerArg</u>	60
<u>GAGGAATGTCCCCAAG</u> CCTTGAGTAGAGTC <u>TCCATCATGGCTGGC</u> AGCCTCACAGGTTG <u>GluGluCysProLys</u> <u>ProLeuSerArgVal</u> <u>SerIleMetAlaGly</u> <u>SerLeuThrGlyLeu</u>	120
<u>CTTCTACTTCAGGCA</u> GTGTCGTGGGCATCA GGTGCCCGCCCTGC ATCCCTAAAAGCTTC <u>LeuLeuLeuGlnAla</u> <u>ValSerTrpAlaSer</u> <u>GlyAlaArgProCys</u> <u>IleProLysSerPhe</u>	180
<u>GGCTACAGCTCGGTG</u> GTGTGTGTCTGCAAT GCCACATACTGTGAC TCCTTGACCCCCCG <u>GlyTyrSerSerVal</u> <u>ValCysValCysAsn</u> <u>AlaThrTyrCysAsp</u> <u>SerPheAspProPro</u>	240
<u>ACCTTCCTGCCCTG</u> <u>GGAACATTTCGGCG</u> TATGAGAGTACACGC AGTGGGCGACGGATG <u>ThrPheProAlaLeu</u> <u>GlyThrPheSerArg</u> <u>TyrGluSerThrArg</u> <u>SerGlyArgArgMet</u>	300
<u>GAGCTGAGTATGGGG</u> CCCATCCAGGCTAAT CACACGGGCACAGGC CTGCTACTGACCCTG <u>GluLeuSerMetGly</u> <u>ProIleGlnAlaAsn</u> <u>HisThrGlyThrGly</u> <u>LeuLeuLeuThrLeu</u>	360
<u>CAGCCAGAACAGAAG</u> TTCCAGAAAGTGAAG GGATTGGAGGGGCC ATGACAGATGCTGCT <u>GlnProGluGlnLys</u> <u>PheGlnLysValLys</u> <u>GlyPheGlyGlyAla</u> <u>MetThrAspAlaAla</u>	420
<u>GCTCTAACATCCTT</u> GCCCTGTCACCCCT GCCCAAAATTGCTA CTTAAATCGTACTTC <u>AlaLeuAsnIleLeu</u> <u>AlaLeuSerProPro</u> <u>AlaGlnAsnLeuLeu</u> <u>LeuLysSerTyrPhe</u>	480
<u>TCTGAAGAACGAATC</u> GGATATAACATCATC CGGGTACCCATGGCC AGCTGTGACTTCTCC <u>SerGluGluGlyIle</u> <u>GlyTyrAsnIleIle</u> <u>ArgValProMetAla</u> <u>SerCysAspPheSer</u>	540
<u>ATCCGCACCTACACC</u> TATGCAGACACCCCT GATGATTCCAGTTG CACAACTTCAGCCTC <u>IleArgThrTyrThr</u> <u>TyrAlaAspThrPro</u> <u>AspAspPheGlnLeu</u> <u>HisAsnPheSerLeu</u>	600
<u>CCAGAGGAAGATAACC</u> AAGCTCAAGATAACC CTGATTCAACCGAGGCC CTGCAGTTGGCCAG <u>ProGluGluAspThr</u> <u>LysLeuLysIlePro</u> <u>LeuIleHisArgAla</u> <u>LeuGlnLeuAlaGln</u>	660
<u>CGTCCCCTTCACTC</u> CTTGCCAGCCCTGG ACATCACCCACTTGG CTCAAGACCAATGGA <u>ArgProValSerLeu</u> <u>LeuAlaSerProTrp</u> <u>ThrSerProThrTrp</u> <u>LeuLysThrAsnGly</u>	720

Mutations made to the Gcc insert are underlined

Figure 4B (continued on next page)

10	20	30	40	50	60
<u>123456789012345</u>	<u>678901234567890</u>	<u>123456789012345</u>	<u>678901234567890</u>		

GGGGTGAATGGGAAG	GGGTCACTCAAGGGA	CAGCCCGAGACATC	TACCACCAGACCTGG		780
AlaValAsnGlyLys	GlySerLeuLysGly	GlnProGlyAspIle	TyrHisGlnThrTrp		
GCCAGATACTTGTTG	AAGTTCCCTGGATGCC	TATGCTGAGCACAAAG	TTACAGTTCTGGGCA		840
AlaArgTyrPheVal	LysPheLeuAspAla	TyrAlaGluHisLys	LeuGlnPheTrpAla		
GTGACAGCTGAAAAT	GAGCCTCTGCTGGG	CTGTTGAGTGGATAC	CCCTTCCAGTGCCTG		900
ValThrAlaGluAsn	GluProSerAlaGly	LeuLeuSerGlyTyr	ProPheGlnCysLeu		
GGCTTCACCCCTGAA	CATCAGCGAGACTTA	ATTGCCCGTGACCTA	GGTCCTACCCCTCGCC		960
GlyPheThrProGlu	HisGlnArgAspLeu	IleAlaArgAspLeu	GlyProThrLeuAla		
AACAGTACTCACAC	AATGTCCGCCTACTC	ATGCTGGATGACCAA	CGCTTGCTGCTGCC		1020
AsnSerThrHisHis	AsnValArgLeuLeu	MetLeuAspAspGln	ArgLeuLeuLeuPro		
CACTGGGCAAAGGTG	GTACTGACAGACCCA	GAAGCAGCTAAATAT	GTTCATGGCATTGCT		1080
HisTrpAlaLysVal	ValLeuThrAspPro	GluAlaAlaLysTyr	ValHisGlyIleAla		
GTACATTGGTACCTG	GACTTTCTGGCTCCA	GCCAAAGCCACCCA	GGGGAGACACACCGC		1140
ValHisTrpTyrLeu	AspPheLeuAlaPro	AlaLysAlaThrLeu	GlyGluThrHisArg		
CTGTTCCCCAACACC	ATGCTCTTGCCCTCA	GAGGCCTGTGTGGC	TCCAAGTTCTGGGAG		1200
LeuPheProAsnThr	MetLeuPheAlaSer	GluAlaCysValGly	SerLysPheTrpGlu		
CAGAGTGTGGGCTA	GGCTCCTGGGATCGA	GGGATGCAGTACAGC	CACAGCATCATCACG		1260
GlnSerValArgLeu	GlySerTrpAspArg	GlyMetGlnTyrSer	HisSerIleIleThr		
AACCTCCTGTACCAT	GTGGTCGGCTGGACC	GAUTGGAACCTTGCC	CTGAACCCCGAAGGA		1320
AsnLeuLeuTyrHis	ValValGlyTrpThr	AspTrpAsnLeuAla	LeuAsnProGluGly		
GGACCCAATTGGGTG	CGTAACCTTGTCGAC	AGTCCCATCATTGTA	GACATCACCAAGGAC		1380
GlyProAsnTrpVal	ArgAsnPheValAsp	SerProIleIleVal	AspIleThrLysAsp		
ACGTTTTACAAACAG	CCCATGTTCTACCAC	CTTGGCCATTCAGC	AAGTTCATTCTGAG		1440
ThrPheTyrLysGln	ProMetPheTyrHis	LeuGlyHisPheSer	LysPheIleProGlu		

Mutations made to the Gcc insert are underlined

Figure 4B (continued on next page)

10	20	30	40	50	60
<u>123456789012345</u>	<u>678901234567890</u>	<u>123456789012345</u>	<u>678901234567890</u>		

GGCTCCCAGAGAGTG GGGCTGGTTGCCAGT CAGAAGAACGACCTG GACGCAGTGGCATTG 1500
 GlySerGlnArgVal GlyLeuValAlaSer GlnLysAsnAspLeu AspAlaValAlaLeu

ATGCATCCCGATGGC TCTGCTGTTGTGGTC GTGCTAAACCGCTCC TCTAAGGATGTGCCT 1560
 MetHisProAspGly SerAlaValValVal ValLeuAsnArgSer SerLysAspValPro

CTTACCACATCAAGGAT CCTGCTGTGGGCTTC CTGGAGACAATCTCA CCTGGCTACTCCATT 1620
 LeuThrIleLysAsp ProAlaValGlyPhe LeuGluThrIleSer ProGlyTyrSerIle

CACACCTACCTGTGG CATCGCCAGTGATGG AGCAGATACTCAAGG AGGCACTGGGCTCAG 1680
 HisThrTyrLeuTrp HisArgGln

NotI
 CCTGGGCATTAAGG GACAGAGTCAGCGAA TTCTGCAGATATCCA TCACACTGGCGGCCG 1740

C 1741

Mutations made to the Gcc insert are underlined

Figure 4B (continued)

10	20	30	40	50	60	
<u>123456789012345</u>	<u>678901234567890</u>	<u>123456789012345</u>	<u>678901234567890</u>			
GGCTCCCAGAGAGTG	GGGCTGGTTGCCAGT	CAGAAGAACGACCTG	GACGCAGTGGCATTG			1500
GlySerGlnArgVal	GlyLeuValAlaSer	GlnLysAsnAspLeu	AspAlaValAlaLeu			
ATGCATCCCGATGGC	TCTGCTGTTGTGGTC	GTGCTAAACCGCTCC	TCTAAGGATGTGCCT			1560
MetHisProAspGly	SerAlaValValVal	ValLeuAsnArgSer	SerLysAspValPro			
CTTACCATCAAGGAT	CCTGCTGTGGGCTTC	CTGGAGACAATCTCA	CCTGGCTACTCCATT			1620
LeuThrIleLysAsp	ProAlaValGlyPhe	LeuGluThrIleSer	ProGlyTyrSerIle			
CACACCTACCTGTGG	CATGCCAGTGATGG	AGCAGATACTCAAGG	AGGCACTGGGCTCAG			1680
HisThrTyrLeuTrp	HisArgGln					
C						1741

Mutations made to the Gcc insert are underlined

Figure 4B (continued)